Author’s response to reviews

Title: The Pubertal Development Mode of Chinese Girls with Turner Syndrome Undergoing Hormone Replacement Therapy

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Author’s response to reviews:

Dear Editor,

We have studied the valuable comments from you and the reviewers carefully, and tried our best to revise the manuscript. The responds to reviewer’s comments are listed as following:

Responds to the reviewer’s comments:

Reviewer 1

Comment 1: Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Response: Thanks a lot for your kind reminding, and we have built the attachments.
Comment 2: The development of response targets to HRT in girls with TS needs further evaluation. This is why the study is principally valuable.

Unfortunately the manuscript as it is lacks sufficient scientific quality. The introduction focuses only on uterine development and potential fertility while half of the project deal with breast development. The reviewer immediately started out to search for reference [1] in the text. It remained also unclear which kind of estrogen was used (<2007 and which one thereafter (giving a reference is not sufficient). The article has a number of other problems with its presentation, not least the unclear language.

Therefore the reviewer concluded regretfully that the article with its interesting topic cannot be recommended for publication. The authors should seek advice from someone who is experienced in scientific writing and in the English language.

Response: Thank you very much for your helpful suggestion. We are pleased to follow the referee’s suggestion.

This study did not show much new result about TS, however we aimed to present the pubertal development with HRT in girls with TS from China, and it is the first report from China to our acknowledgement. We also discussed it with comparison to that in other countries, so our study was mostly a descriptive analysis and research with scientific quality.

The reference [1] seems lost during the repeated revise before submission for our carelessness, and we have added it up in the manuscript.

All the estrogen used in our study was estradiol valerate, and we had made it clearer by clarifying it in the beginning of Method section, line 102-103, 106-107.

We apologized for our poor English. We tried our best to improve it in revision and listed the detailed modified lines at end of this word document. Actually, we had considered about it, so we had our manuscript edited by Nature Research Editing Service before submission, and we also have the certification offered from the company. But it is not a guarantee of poor language, so we are also willing to edit our manuscript again to make it better if it still necessary.

Thank you again for your attention to our manuscript.
Beata Wikiera (Reviewer 2): The work takes up an important matter of optimization of the treatment of primary hypogonadism in Turner syndrome. However, the methodology of conducted surveys raises a number of concerns.

My concerns mainly refers to the methodology. The authors write about two decades of retrospective research without any information on the methods used to measure the levels of hormones and their results. The question arises if they have not changed over the period of the 20 years?

Response: Thank you for your careful reviewing to our manuscript. According to the comments, the manuscript has been revised extensively and shown below. The modified parts have been marked by red in the revised manuscript.

We also worried about the same problem with the respective referee, so we discussed with the director of laboratory center in our hospital. We identified that chemiluminescent microparticle immunoassay was used to measure the levels of FSH and LH in the past 20 years, and the kits were bought from the same company, Abbott Laboratories. At the same time, the laboratory center also adjusted the reference ranges every time the lot number of kit changed, to make sure the value could be compared with the value published with the kit of previous lot number. We did ignore to describe it in the text, and the detail information about the measurement of serum FSH and LH level was added in the text, line 121 - 122.

The same problem concerns equipment used in ultrasound examination of uterus. The question is whether the same equipment has been used in the examination of all patients and if it has been performed by the same doctor?

Response: Thank you for your prompt attention to our manuscript and thanks a lot for your helpful suggestions. We are pleased to follow your criticism and the manuscript has been extensively revised to your suggestions. The corresponding explanations of each point which is raised in your comments as follows:

The reason we studied our data within the recent 20 years is that the duration we had the ultrasound image recorded in our hospital is 20 years. We invited an ultrasonologist to review the ultrasound image, and we had made it clearer in line 127 - 128. We did not include ultrasonologist as one of authors for some reasons, but we have supplemented this in the acknowledgement part in line 356 - 358.
Methodology:

71 According to the recent guidelines published in EJE in 2017 (not cited by the authors) there is a recommendation of diagnosing TS in phenotypic females with a karyotype containing one X chromosome and complete or partial absence of the second sex chromosome, associated with one or more typical clinical manifestations of TS. The authors has not stated if all patients had clinical manifestations of TS.

Response: It is our carelessness not to mention it. Following your professional advice, we had made it clearer in line 83 – 84.

77 The description of structural anomalies of X chromosome should be included.

Response: Following your advice, we have added it in line 89 – 93.

82 Was the beginning of pubertal development at the age of 12 spontaneous in all cases? If so what decided about pharmacological treatment?

Response: Thanks a lot for your attention. It is our ignorance not to make it clear. 1) It means that the girls with TS who initiated HRT no less than 12 yrs could be included into this study, so the girls younger than 12 yrs could not be included, because they may face the spontaneous pubertal development. The girls we included to detect the breast development in this study aged 15.82 (14.05, 17.59) years old. We have tried to make it clearer by changing the words “at” to “no less than” in line 97 -98. 2) The clinical treatment of beginning the pubertal induction at about 12 years old was given according to recommendation from guideline published in 2007 and 2017, and before 2007, we did it by doctor’s expertise, and only the girls began HRT after 12 yrs could be included in this study.

87 Are there any data on the age of girls with TS at the moment of starting treatment? The authors do not specify drugs used in HRT therapy though stating their doses.

Response: It is our fault not to describe it well. 1) We mentioned the age of girls with TS in the Result section, in line 164 – 167, the 71 girls with TS began HRT at 15.71 ±1.73 yrs of age, with a range from 11.5–19.75 yrs. The median age of the 63 TS patients in the breast research project at the beginning of puberty induction was 15.82 (14.05, 17.59) yrs. 2) All the estrogen used in our study was estradiol valerate, and we have made it clearer by clarifying it in the beginning of Method part, in line 102 - 103.
The article does not precisely mention general information concerning: initial weight, BMI, FSH, LH, estradiol. A table would explain the problem.

Response: It is our ignorance not to make it clear in the text, we had put the initial weight, height, FSH, LH and estradiol in table 3, line 475. BMI was described in the text, line 174 - 175.

The authors write only about 39 patients though earlier they give number 63. What happened to over 20 of them during the research?

Response: Thank you for your attention on this problem. It is a little bit confused because this study is a retrospective study. Though we included 63 girls with TS, but only 39 of them reached the Tanner breast stage of B5, and that was why we had only 39 girls when we were discussing about the ‘final breast stage’. The fact of 39 in 63 patients reached stage B5 was also a result on breast development in this study.

At the same time, we still want to explain the reason why we cannot keep the number of each Tanner breast stage the same in Table 1, because some patients would face a change of two stages in one follow up duration. For example, a patient was identified as Tanner stage B2, and then she was identified as B4, not B3 after 6 months when she came back to our center, so we lost the data of this patient in B3, and that was inevitable cause it is hard for us to keep every patient back to hospital every 3 months or even shorter time. We have described it in the footnote of Table 1, and we are willing to add it in the text if it is necessary.

Why the development of breast and uterus is observed in two different groups of patients?

Response: Thank you for your careful reviewing. Actually, the development of breast and uterus is observed in the same group of 71 patients, but 8 of them used HRT less than 1 year when we analyzed the data, so we excluded them for our inclusion criteria.

What is a precise division according to the age of control patients who served the observation of uterine development?

Response: We analyzed the uterine development of girls with TS according to Tanner breast stages, so we also described the control group according to Tanner breast stages for a consistent division standard. We did not elevate them by age, so we did not describe the precise division according to the age of control patients.
Results:

141 There are inconsistencies concerning the beginning of puberty in patients with TS. In the methods section it is 12 years, in the results section it is 15.71 years.

Response: We felt so sorry about the way we described in methods section, and we meant that we included the girls who began HRT no less than 12 years for analysis of breast development. We have revised it in line 97 -98.

145 Why in the control group the mean age is given in the methods section and median age in the result section?

Response: We should have described it clearly. These two groups of age are different. The data we mentioned in the methods section was the baseline age, which means the age of Tanner breast stage B1 in those 139 girls. However, the data we mentioned in the result section is the age of girls who reached B2 (it means the entry in to puberty in the 139 girls). The reason we mentioned the age of B2 in control group was that we cannot capture information accurately about the duration from B1 to B2 in control group of normal girls.

158 What drug, what dose and what way of treatment was offered to the patients at the stage of B3, B4, B5? Were there any differences?

Response: Thank you a lot again for your careful reviewing, and we have revised it clearer in line 102 – 103, 106 - 108. The detail dose and duration of estradiol valerate was mentioned in table 2.
Conclusions are the repetitions of survey results. Published works confirm the efficiency of HRT, thus the article does not bring any added value. Fast growth of breasts and uterus at the beginning of treatment can indicate too quick increase of estrogens doses. Unfortunately, a scheme of conduct is not presented in details. The work requires substantial amendments.

Response: 1) We know that most of our conclusion was similar to the published works, but this study was about HRT in girls with TS from China, and it was not published before to our knowledge. The additional aim of this study was to improve diversity of the TS database. Beside those, there are also differences in our study even with the similarities. For example, the breast development in girls with TS was not so sensitive to HRT in China and only half of them reached stage B5, and we discussed it in discussion section. We also found that 1/LH may relate with the potential of reaching breast stage 5 in girls with TS. At last, we proposed the main attention of HRT in girls with TS should focus on Tanner breast stage B2-4 other than B5. It may give some hints to the proposal of optimal HRT regimen. 2) The scheme of the conduct was described in methods section and summarized in Table 2 meanwhile, although we did not describe it in detail for long duration and large amount of subtle dose adjustment during follow-up, and we hope it could be appreciated by kind referees.

Not all references were cited

Response: Thanks for reminding us and it is all due to our carelessness. Some of the references may be lost during repeat revision before submission and we have checked it carefully in text.

Poor English

Response: We apologize for our poor English. We tried our best to improve it in revision to present it clearer. The parts we modified in the text are listed here and pointed out with red highline font in the text.

The parts we revised are listed as follow:


We corrected some information that was described falsely. We revised it in line 47-49, 56-57, 210, 212, 307. When we were revising our manuscript, we found the abstract parts should be B2-4 other than B2-3, because the uterine volume in B4 was also significantly greater than that in B3. All corrected lines listed above are for the same reason.
The citation 9 and 10 were showed not validated, and the citation 5 was not checked.


We had checked the figure we submitted in the submitting system.

These above are the response to reviewers. We feel so grateful again to reviewers and editors for your attention to our manuscript. It would be highly appreciated if you are kind to give a favorable consideration on it.